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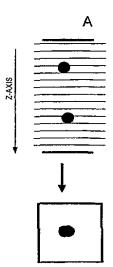
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[Continued on next page]

(54) Title: SIGNAL COUNTING FOR IN SITU HYBRIDIZATION



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(57) Abstract: Fluorescently tagged nucleic acid probe signals are counted in biological specimens by determining a ratio of signals from a test probe to signals of a reference probe. Probe signals need not be counted with reference to cells, nuclei, or nuclear contours. Gene amplification or deletion can thus be detected by analyzing the ratio. Successive image slices are obtained by confocal microscopy, and the images are digitized. The digital images are transformed and analyzed to combine contiguous fluorescent signal segments in successive optical sections to identify discrete probe signals, or spots. Spots overlapping in the axial and transverse dimensions of a three-dimensional representation of the biological specimens can be distinguisghed. A graphical user interface presents various features for consideration by a user, who can provide guidance to a computer system counting the spots. Various features directed to identifying spot clusters and autofluorescent material can increase accuracy of spot counting.



patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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?NATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12Q1/68 G01M G01N15/14 According to International Patent Classification (IPC) or to both national classification and IPC Minimum documentation searched (classification system followed by classification symbols) IPC 7 C120 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE C. DOCUMENTS CONSIDERED TO BE RELEVANT Relevant to claim No. Citation of document, with indication, where appropriate, of the relevant passages 1,6-23,RODENACKER ET AL.: "Groping for quantitative digital 3-D image analysis: 27-40, 48-63 an approach to quantitative fluorescence in situ hybridization in thick tissue sections of prostate carcinoma" ANALYTICAL CELLULAR PATHOLOGY, vol. 15, no. 1, 1997, pages 19-29, XP001024113 1-26, Υ the whole document 41-47 WO 99 44063 A (THE UNITED STATES OF 1-5 X AMERICA) 2 September 1999 (1999-09-02) page 13, line 16 -page 24, line 20; 1-14. 24-26, examples 1.3 41-47 -/--Further documents are listed in the continuation of box C. X Patent family members are listed in annex. Special categories of cited documents: *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the "A" document defining the general state of the art which is not considered to be of particular relevance earlier document but published on or after the international "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu-ments, such combination being obvious to a person skilled citation or other special reason (as specified) 'O' document referring to an oral disclosure, use, exhibition or other means in the art. document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of mailing of the international search report Date of the actual completion of the international search 21/09/2001 17 September 2001 Authorized Officer Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentiaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016

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Botz, J

IN. INATIONAL SEARCH REPORT

Inter. onal Application No PCT/US 00/25465

		PCT/US 00/25465						
C.(Continu	Rinuation) DOCUMENTS CONSIDERED TO BE RELEVANT							
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.						
(AUBELE ET AL.: "Comparative FISH analysis of numerical chromosome 7 abnormalities in 5-um and 15-um paraffin-embedded tissue sections from prostatic carcinoma" HISTOCHEMISTRY AND CELL BIOLOGY, vol. 107, no. 2, 1997, pages 121-126, XP001024090	1,6-23, 27-40, 54-63						
	the whole document	1-14, 24-26, 41-47						
,	US 5 817 462 A (GARINI ET AL.) 6 October 1998 (1998-10-06) column 26, line 45 - line 58	1-23						
-								

IN .RNATIONAL SEARCH REPORT

Information on patent family members

inten Juai Application No PCT/US 00/25465

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 9944063	A	02-09-1999	AU AU EP EP WO WO	2875799 A 2973599 A 1068528 A2 1066517 A1 9944062 A1 9944063 A2	15-09-1999 15-09-1999 17-01-2001 10-01-2001 02-09-1999 02-09-1999
US 5817462	A	06-10-1998	US US US US US US US US US US US US US U	5936731 A 5784162 A 5539517 A 2730597 A 29724412 U1 0896631 A1 2000509977 T 9740191 A1 6066459 A 5719024 A 5856871 A 5835214 A 5798262 A 6018587 A 5912165 A 5906919 A 0832417 A1 121425 A 11503239 T 9722848 A1 6055325 A 0830564 A1 121426 A 11500832 T 6075599 A 9721979 A1 6007996 A 6165734 A 5991028 A 6198532 B1 0767361 A2 3033470 T3	10-08-1999 21-07-1998 23-07-1996 12-11-1997 22-03-2001 17-02-1999 08-08-2000 30-10-1997 23-05-2000 17-02-1998 05-01-1999 10-11-1998 25-08-1998 25-01-2000 15-06-1999 25-05-1999 01-04-1998 13-08-2000 23-03-1999 26-06-1997 25-04-2000 25-03-1998 29-02-2000 19-01-1999 13-06-2000 19-06-1997 28-12-1999 26-12-2000 23-11-1999 06-03-2001 09-04-1997 29-09-2000